

6.

Introgressive Hybridization in Domesticated Fishes.

I. The Behavior of Comet A *Platypoecilus maculatus* Gene in *Xiphophorus hellerii*.MYRON GORDON.¹

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(Plates I-III).

The platyfish's comet gene PC^o was found in 1932 among the wild population of *Platy-poecilus maculatus* in a tiny pool within the bed of a tributary stream of the Rio Tonto in the State of Oaxaca, Mexico. In 1939 two more populations containing the PC^o gene were found in the Rio Jamapa, Veracruz, and in several of the Rio Papaloapan tributaries in Oaxaca. The comet is one of a galaxy of genes in the natural populations of the platyfish, the most variable vertebrate, with respect to color pattern polymorphism, in North America. The comet gene PC^o belongs to an autosomal series of seven, dominant, multiple alleles, which include onespot, P^O ; moon, P^M ; moon complete with satellites, P^{Mc} ; crescent, P^C ; crescent complete, P^{Cc} ; twinstripe, P^T ; and the universal recessive +.

During the study of this series of wild alleles, the comet-marked platyfish was mated in some instances to members of its own wild population and then, in contrast, to a number of domesticated breeds of the same species. A comparison of the results of these two types of matings was quite startling and unexpected. An entirely new pattern was created, known popularly among fish fanciers as the *black wagtail*. It resembles the color scheme in the Himalayan rabbit and Siamese cat, in which the extremities are much darker than other parts of the body.

Owing to the ease in which *P. maculatus* hybridizes with *Xiphophorus hellerii*, a mating between a wild comet platyfish and a wild swordtail was attempted and accomplished. As a result, the hybrids carrying the comet gene showed up with the same wagtail response. By repeated backcrosses of the wagtail platy-swordtail hybrid to the swordtail, a stock was developed indistinguishable from the *X. hellerii* configuration but containing the PC^o gene of *P. maculatus*. The wagtail swordtail so produced is now

well established in the trade devoted to aquarium fishes.

Every step in the production of this new wagtail swordtail is known in this instance, and this will be described in detail. There are many colorful swordtails on the aquarium fish market most of which, if all the facts were known, could be traced back to one or more wild platyfish genes introduced into the swordtail by the process of introgression. This type of hybridization is the basis, also, for the development of melanomas in platyfish-swordtail combinations. Essentially the malignant tumors depend upon the interactions of the platyfish genes for macromelanophores Sp or N in association with a number of swordtail genes. With every backcross of the melanotic hybrid to the swordtail, the characteristic contours of the swordfish are more closely approached, while at the same time the severity of the melanoma is enhanced.

The process of introgression is often reversed; that is, a few swordtail genes may modify the basic platyfish genes. This is probably the explanation for the wagtail platyfish and for a number of the brilliant red, black and other domesticated varieties of the platyfish.

THE COMET PATTERN.

The comet is a simple pattern in wild platyfish. It consists of two straight black lines, one on the upper and one on the lower border of the tail fin. These dual black streaks are not parallel but flare slightly in conforming to the broadening of the caudal fin. This has the appearance of a conventionalized comet's train. A microscopic examination of the black lines of the comet reveals that they are composed of hundreds of tiny pigmented cells or micromelanophores concentrated along the upper and lower margins of the tail fin. The rest of the fin, in contrast, is transparent although there are scattered micromelanophores over all of it. The pigmented cells of the comet,

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of the tail fin proper, and of the body proper are similar morphologically. These micromelanophores are similar, too, to the pigmented cells which compose the other six patterns of the tail and caudal peduncle. When the micromelanophores are scattered, they produce an olive gray appearance; when concentrated they produce a jet black effect.

THE COMET REACTION IN WILD POPULATIONS.

Two distinct geographical populations of *P. maculatus* containing the comet platy were tested. The Rio Tonto population belongs to the Rio Papaloapan drainage system and in this system the comet gene is present in 8% of all platyfish. The second group belongs to the Rio Jamapa system, being collected at Plaza de Agua near El Tejar, a few miles west of the city of Veracruz. This area marks the most northern limit of the species as far as known; here 5% of the platyfish population carry the gene PC^o . The same species is known from two other great river systems, the Rio Coatzacoalcas and the Rio Usumacinta, but no comets have been reported, as yet, from them. An analysis of speciation in this fish by the use of pattern gene frequencies, including PC^o , is being prepared by Gordon (1946a).

In the paper just mentioned genetic evidence will be presented showing the relation of the PC^o gene to the others of its allelic series. When a comet platy is mated to any other member of its wild population, their offspring will display the comet pattern unchanged, regardless of the presence of any of the other markings. Natural combinations such as PC^oPO , PC^oPM , PC^oPMe , PC^oPC , PC^oPC^o and PC^oPT have been observed. In addition, single and duplex combinations have been seen with the sex-linked genes: Sp , Sr , Sd and N in more complex arrangements of patterns. However, no matter what other wild patterns a platyfish may have, the comet, if present, is visible and remains phenotypically unchanged.

THE COMET REACTION IN DOMESTICATED POPULATIONS.

When a wild platyfish carrying the comet gene was mated to several different domesticated breeds of the same species, the F_1 hybrids carrying PC^o showed an entirely new phenotypic expression of it. When quite young the comet hybrids appeared in their conventional pattern, but as they continued their growth, the intensity of the black pigmentation of the upper and lower margins of the tail fin spread to the intermediate regions of that fin. At the same time the dorsal, the anal, the ventral and even the pectoral fins became darker too. Finally when the hybrid fish reached maturity, the micromelanophores in great concentrations blackened all the fins, and in addition they darkened the

upper and lower jaws and the edges of the operculum. Expressing this reaction in short: all extremities were darkened. The resulting pattern is somewhat similar to the color scheme seen in Siamese cats, Himalayan rabbits, great Dane dogs, Dorset sheep and others in which the exposed parts, the muzzle, the ears, the digitary areas and the tail are strikingly darker than the rest of the body.

The action of the gene PC^o for comet was apparently modified in F_1 hybrids by a gene or genes contributed to the hybrid by the domesticated platyfish. The reaction of the comet with its modifier was first detected when the comet fishes of the Rio Papaloapan system were mated to the aquarium-bred variety known as the goldplaty. Later the same reaction was rediscovered when a wild comet platy from the Rio Jamapa was mated to several domesticated varieties.

THE GENETIC NATURE OF THE COMET MODIFIER.

In *P. maculatus*, prior to the discovery of the PC^o modifier gene, E , three linkage groups were known: a sex-linked group with seven dominant genes, an autosomal allelic series with seven dominant genes and another autosomal group containing the one "domesticated" recessive golden, st . This platy has 24 haploid chromosomes according to Ralston (1934) and Friedman and Gordon (1935).

The following is an analysis of the genetic association of the E gene. The presentation of the following data and their analyses are not necessarily given in the chronological order in which the experiments were conducted.

$$\begin{array}{ccc} \text{"Domesticated"} \text{ } \varnothing & & \text{"Wild"} \text{ } \delta \\ 1. \quad \frac{Dr \text{ } st + E}{+ \text{ } st + E} \times & \times & \frac{+ + PC^o +}{+ + PC^o +} \end{array}$$

In the above and succeeding formulae of genetic constitutions, Dr represents the gene for a red-dorsal fin, shown by Kosswig (1931) to be dominant and sex-linked. This was confirmed independently by Gordon (1931) who at one time used the symbol Rf for this gene. In domesticated females its chromosome associations are $(Z)Dr (W)+$.

The recessive st represents few or no micromelanophores and the absence of these black cells reveals the many underlying xanthophores which give this fish variety its golden coloring.

PC^o represents the comet pattern and E refers to its specific modifier which, as will be seen, is present in most domesticated stocks in a homozygous state. The $+$ refers to universal recessive of the allelic series and to the "neutral wild type."

Together with this first mating, two others may be presented and discussed at the same

time because in each case the female parents had the same genotypes, and the males, too, were for the most part similar genetically for the characters under analysis. The second male differed only in that the PC^o gene was associated with another one of the dominant alleles, the gene P^o for onespots while the third male had the allele PC for crescent as its PC^o associate. Their genotypes are given below:

Females

2. $\frac{Dr\ st + E}{+ \ st + E} \times \frac{+ + \frac{PC^o}{+} +}{+ + \frac{P^o}{+} +}$

Males

Females

3. $\frac{Dr\ st + E}{+ \ st + E} \times \frac{+ + \frac{PC^o}{+} +}{+ + \frac{PC}{+} +}$

Males

It is clear from the results indicated in Table I that the sex-linked factor Dr was transmitted from the mothers to all their sons in the conventional manner according to (Z) Dr (W) + chromosome sex-determining mechanism. But Gordon (1946c) has indicated that the wild platyfish male has the XY chromosome arrangement. Thus it may seem from these experiments that among the F_1 , (X) + (W) + and (Y) + (W) + are female while (Z) Dr (X) + and (Z) Dr (Y) + are male. A further discussion of this phase of the problem in matings of "wild" and "domesticated" platyfish is treated in another paper by Gordon (1946b).

All the F_1 of the three matings were olive gray, showing complete and uniform dominance of the "wild" St (or +) over the recessive "domesticated" golden gene st .

The first "wild" male comet platy (1-38) was apparently homozygous for PC^o as all of the F_1 had this factor but its phenotype ex-

pression modified by extensor gene E was changed into the wagtail pattern.

The offspring of the second (1-39) and the third (1-40) males show that the presumed modifier E acts on PC^o but not on P^o or PC , and we have additional data at this time showing that E does not act on PC^c , P^M , P^{Mc} or PT , the remaining members of the series, and E has no discernible phenotype by itself. Only the combination $PC^o E$ produced the wagtail reaction.

Matings 1, 2 and 3, taken individually or together, clearly show that E is not sex-linked, for while Dr is transmitted from the mother to her sons only the wagtail reaction $PC^o E$ is visible in the sons and daughters.

Further evidence of the autosomal nature of the PC^o modifier may be seen in matings listed in Table II. In mating 4 the broods of two genetically similar females were pooled, and the observed results are apparently consistent with the theoretical values expected if E is considered independent of the two known autosomal factors in the mating, PC^o and St . Previous work (Gordon, 1927) has shown that the number of golden st is usually deficient, the deficiency being due to differential viability of st in contrast with the wild allele St . If E were linked to PC^o we might have expected that the ratio between $PC^o E$ and E would have been less than 3:1, since linkage, if it existed, would have been in the repulsion series; actually the number of $PC^o E$ was slightly greater than expected on the basis of a 3:1 ratio.

The independence of E and PC^o with respect to their linkage relations is clearly demonstrated in mating 5. Here approximately equal numbers of modified comets $PC^o E$, and non-comets, + E , (20:15) were found, whereas if E were linked with PC^o ,

TABLE I.

MATING No.	1		2		3	
CULTURE No.	31		32		33	
P ₁ PEDIGREE No.	♀ 9A1	♂ 1-38	♀ 9A2	♂ 1-39	♀ 9A3	♂ 1-40
P ₁ PHENOTYPES	Dr st + E	+ + P ^{co} +	Dr st + E	+ + P ^{co} P ^o +	Dr st + E	+ + P ^{co} P ^c +
F. PHENOTYPES	♀	♂	♀	♂	♀	♂
Dr + P ^{co} E	0	12	0	5	0	14
+ + P ^{co} E	9	0	6	0	10	0
Dr + P ^o E			0	7		
+ + P ^o E			8	0		
Dr + P ^c E					0	10
+ + P ^c E					15	0
Totals	9	12	14	12	25	24
χ ² values	0.428		0.768		1.693	

TABLE II.

MATING	4		5		6		7	
CULTURE NO.	53		59		55		54	
P ₁ PEDIGREE NO.	♀ 31-1, 2	♂ 31-11	♀ 9A2-1	♂ 31-11	♀ 31-3, 4	♂ 12-11	♀ 53-1	♂ 53-11
P ₁ PHENOTYPES	+ + P ^{Co} E	Dr + P ^{Co} E	Dr st + E	Dr + P ^{Co} E	+ P ^{Co} E	+	st P ^{Co} E	st P ^{Co} E
F ₁ PHENOTYPES	♀	♂	♀	♂	♀	♂	♀	♂
Dr + P ^{Co} E	14	8	3	6				
+ + P ^{Co} E	13	12	4		14	11		
Dr st P ^{Co} E	3	2	2	4				
+ st P ^{Co} E	2	1	1				14	18
Dr + P ^{Co}	4	5						
+ + P ^{Co}	4	3			11	8		
Dr st P ^{Co}	1	0						
+ st P ^{Co}	0	1					4	6
Dr + + +	6	8	2	5				
+ + + +	3	2	4		28	24		
Dr st + +	2	1	1	3				
+ st + +	1	0	0				8	8
Totals	53	43	17	18	53	43	26	32
χ ² values	12.764		8.267		1.417		0.237	

then one might have expected only one *P^{Co} E* in four individuals (1:3). Independence of these genes is fully substantiated by the results of mating 6, where the male parent, being a "wild" individual and presumably homozygous recessive for the *P^{Co}* modifier *e*, produced when mated to an F₁ *P^{Co} E* approximately equal numbers of *P^{Co} E* and *P^{Co} +*. If *P^{Co}* were closely linked to *E*, no *P^{Co} E* would have been expected; if linked but separated moderately, a few *P^{Co} E* might have been expected as crossovers; but the data (25:19) indicate independence. The results shown in mating 7, where golden wagtails (*P^{Co} E*) were inbred, indicate a similar conclusion.

The data presented in Table III indicate further evidence that domesticated stocks of *P. maculatus* carry *E* (the dominant modifier of *P^{Co}*), in a homozygous state. All four golden platyfish of culture 9A listed in Table II were of this type; and the spotted female, 8 C1, and golden male, 9A21, listed in Table III, were homozygous for *E* too. In mating 8, the spotted female carried the pattern gene on its W chromosome, (Z)+ (W)*Sp*. This accounts for the mother-to-daughter type of inheritance of the *Sp* gene. (Again although XY represents the P₁ wild male (No. 1-39), in the F₁, XW and YW apparently determine femaleness and ZX and ZY determine maleness). Again, *E* apparently has no effect on the phenotypic expression of the allele *P^{Co}*.

Further consideration of the sex-linked

factors *Sp* and *Dr* in linkage relations to *E* seem unnecessary. Additional data are available of the linkage independence of *E* with respect to *St* and *P^{Co}*. In matings listed under No. 9 representing broods 46, 47 and 48, there were 146 *P^{Co} E*, 41 *P^{Co} +*, and 55 ++. The theoretical expectancy if *P^{Co}* and *E* are independent is as follows: 125 *P^{Co} E*, 45 *P^{Co} +*, and 60 ++, which is a significantly good fit. The X² value in this mating results with regard to *st*, *P^{Co}* and *E* is P = 31.

The results of mating 9 show that it is not likely that *E* is linked to *St* for when *P^{Co} E* was crossed with + *P^{Co} E*, 12 were *st P^{Co}* out of 242 offspring. On the basis of three independent factors 11.4 or 3/64 of the total were expected theoretically. All the other phenotypes appear in proportions appropriate to the theoretical values expected upon the same basis.

Further confirmation of the independence of *St*, *P^{Co}* and *E* is presented in the results from matings 10 and 11. If *P^{Co}* and *P^{Co}* were linked to *E*, one ought not to get any *P^{Co} P^{Co} E* in mating 11, yet 19 were observed; and 17 would be expected on the three independent factor basis. If *E* were linked to *St*, no *st P^{Co}* or *P^{Co} P^{Co} E* would be expected, yet both types were present.

THE DISTRIBUTION OF THE *E* GENE IN XIPHOPHORIN SPECIES.

Tests for the presence of the *E* gene in some of the wild xiphophorin species were

TABLE III.

MATING No.	8		9		10		11	
CULTURE No.	44		46-47-48		49-50		51-52	
PEDIGREE No. GENOTYPES	♀ 8C1 Sp st + E + st + E	♂ 1-39 ++ P ^{Co} + ++ P ^O +	♀ 44-1,2,3 + P ^{Co} E st + +	♂ 44-11,12 + P ^{Co} E st + +	♀ 44-4, 5 + P ^{Co} E st + +	♂ 9A21 ++ E ++ E	♀ 44-6, 7 + P ^O E st + +	♂ 44-13 + P ^{Co} E st + +
F. PHENOTYPES	♀	♂	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
Sp + P ^{Co} E	7	0						
+ + P ^{Co} E	0	7						
Sp + P ^O E	6	0						
+ + P ^O E	0	9						
+ P ^{Co} E			121	101.8	28	22.8	14	12.6
st P ^{Co} E			25	34.2	21	22.8	3	4.2
+ P ^{Co} +			29	34.2			5	4.2
st P ^{Co} +			12	11.4			2	1.4
+ +			42	45.4	24	22.8	15	16.8
st +			13	15.2	18	22.8	4	5.6
+ P ^{Co} P ^O E							17	12.6
st P ^{Co} P ^O E							2	4.2
+ P ^{Co} P ^O +							2	4.2
st P ^{Co} P ^O +							1	1.4
+ P ^O							20	16.8
st P ^O							5	5.6
Total	13	16	242	242.2	91	91.2	90	89.6
X ² values	0.812		7.163		1.265		5.583	

made by mating wild comet-carrying *P. maculatus* to *Xiphophorus hellerii*, *P. xiphidium* and *P. couchianus*. Tests were made also in three domesticated varieties of the swordtail: the albino, the red, and the golden tuxedo.

In Table IV the various interspecific matings are listed. The first-generation hybrids between *X. hellerii* and *P. maculatus* are designated as *XP*. When this hybrid is backcrossed to *X. hellerii*, the offspring are indicated as *XPX*, and when *XPX* is backcrossed again to *X. hellerii*, their offspring are designated as *XPXX*. The *XPXX* hybrids are not all alike, nor would they all pass as swordtails (see discussion). In 12 to 15, the results of crossing four wild *P. maculatus* comets, *P^{Co}*, with two albinos, one red and one wild female *X. hellerii*, show that the *E* modifier was present in a homozygous state in all of the swordtails, since the wagtail reaction rather than the unmodified comet pattern appeared in half of the hybrids. When the hybrids of mating 12 were inbred (mating 16), the number of wagtails (*P^{Co} E*), comets (*P^{Co}*), and neutrals (+) showed up approximately in the ratio of 9:3:4. This ratio represented the distribution of the three phenotypes in matings 16 and 17, too. Summing matings 16

through 18, there were 70 *P^{Co} E*, 15 *P^{Co}*, and 37+, while the expected ratio was 68.4 *P^{Co} E*, 23 *P^{Co}*, and 30.4 +. This summary does not, of course, include a number of presumably *P^{Co} E*, *P^{Co}*, and + in combination with the homozygous recessive *ii* for albinism, because the melanin pigment inhibitor *i* suppresses all melanic patterns. The fact that the albinos appear in far fewer numbers than the theoretical expectancy, has previously been noted by Gordon (1942).

When the species hybrids were backcrossed to the swordtail in an effort to recreate the body configuration of the swordtail and at the same time to retain the wagtail pattern (matings 19 through 23, and 25), all the domesticated and wild *X. hellerii* used proved to be homozygous for the *E* modifier in the backcross generation. When, however, the *F*₁ hybrid was backcrossed to a wild *P. maculatus* which introduced the non-modifier (mating 24), an 8 *P^{Co} E* to 4 *P^{Co}* to 10 + ratio was observed, and 5.5 to 5.5 to 11.0 ratio was expected on the basis of 1:1:2 ratio and 22 individuals.

With each backcross of the wagtail platy-swordtail hybrid to the *X. hellerii*, the resulting broods contains individuals that approach the swordtail more perfectly in body type; but there are many swordtail-platyfish

hybrids in the *XPXX* category that have many combinations of hereditary factors; some of them indeed may be compared to the F_1 or *XP*.

When a comet *P. maculatus* was mated with a *P. xiphidium*, the wagtail reaction was weakly evident. A similar *P. maculatus* mated with *P. couchianus* produced a more definite wagtail hybrid. Since one mating each was tried, the distribution of the *E* gene in xiphophorins other than *X. hellerii* is still incompletely known.

DISCUSSION.

THE CONDITIONS IN NATURAL POPULATIONS.

Wild swordtails are quite uniform with respect to color patterns whereas wild platyfish are extremely variable. In over 10,000 specimens of both species taken over their entire range in southern Veracruz and Oaxaca in Mexico and in British Honduras and Guatemala, not a single hybrid was found, yet at eight localities in this range the two species may be found side by side. Some of the isolating mechanisms such as psychological and ecological have been suggested previously by Gordon (1943, 1946a) to account for lack of natural hybridization. In the laboratory or home aquaria these barriers to hybridization are easily overcome. In a historical account of the early domestication of these species Gordon (1934) has found that the platy was first imported, probably from the Rio Coatzacoalcos, in 1907, and the swordtail followed in 1909, probably from the same river. Only two years later, 1911, hybrids between the species were recorded by aquarists writing in the *Blätter* and in the *Wochenschrift für Aquarien und Terrarienkunde*.

At present the tropical-fish dealers' catalogues list a dozen or more color varieties of the swordtail, only one of which, the albino, may have definitely arisen by the process of mutation. The other domesticated varieties, including such types as golden, red, black, tuxedo, "montezuma helleri" and their combinations, may be traced to introgressive hybridization of the many individual types of platyfish with the swordtail. Since the wagtail swordtail was produced in the laboratory, every step in its development has been recorded. Evidence of the origin of many other domesticated swordtails is now available also and will be presented in a series of papers.

The introgressive process is much clearer in the development of domesticated swordtails than in domesticated platyfish, but the same process has been going on in both. It seems to be clearest in the production of the wagtail platy. Since wild *P. maculatus* do not carry the *E* factor and wild *X. hellerii* do, it is likely that *E* was transferred to domesti-

cated platies during the many matings made between these species.

An alternative suggestion may be made in accounting for the presence of the *E* factor in domesticated stocks. The early importations of platyfish were probably made from the Rio Coatzacoalcos, according to the analysis made by Gordon (1946a). The *PCo* factor does not occur (as far as our data show) in the platyfish population of this river but the *E* factor may be present and it may have been transmitted in domesticated stocks generation after generation from 1907 to the present time. The former suggestion seems more plausible at this time.

MODIFYING FACTORS AND QUANTITATIVE CHARACTERS.

From his analyses of natural fish hybrids and particularly from his laboratory studies of species hybrids in *Mollienia* and related forms, Hubbs (1940) has become convinced that in species hybrids systematic characters of fishes generally show blending inheritance and that simple Mendelian segregation very seldom results; and this seems to apply to hybrids of subspecies and races. Hubbs finds that the characters which distinguish species and subspecies behave in hybridization experiments "in such strict conformance with the Galtonian scheme of inheritance that one can, for instance, compute rather precisely the number of dorsal rays in the final multiple hybrid by striking theoretical averages through the complex mating chart, starting only with the known average value of each form as it is introduced into the multiple matings. Such characters as position of fins, form of body and coloration appear to show a similar type of inheritance."

Kosswig (1931-39) and Gordon (1931-37) have long held that multiple genetic factors may account for the striking modifications in coloration in species hybrids between a particular *P. maculatus* and *X. hellerii*. Gordon (1937), for instance, suggested that the swordtail carries two sets of dominant modifying factors which act upon the spotting (*Sp*) gene of the platyfish. *Sp* is the gene that governs the development of specific pigment cells, the *macromelanophores*, which produce an irregular spotted pattern—a normal condition found in wild stocks as well as in domesticated ones. Thus the following formulae were used to represent the species:

Swordtail × Platyfish
++ AA BB × SpSp ++ ++

The first generation hybrids are all *Sp+* *A+* *B+*. The interaction of *A* and *B* with *Sp* produces a state of melanosis which frequently is so intense that it leads directly to the development of melanomas.

The reality of these modifiers *A* and *B* may be evaluated in the two opposing types

of backcrosses, one to the platyfish, the other to the swordtail:

BACKCROSS OF F_1 HYBRIDS.

To the Platyfish

Spotted platyfish \times Melanotic hybrid

$Sp+ ++ ++ \times Sp+ A+ B+$

Backcross hybrids

NORMALS	MELANOTIC
$SpSp ++ ++$	$SpSp A+ B+ (2)$
$Sp+ ++ ++$	$Sp+ A+ B+ (2)$
$++ A+ B+$	$SpSp A+ ++ (1)$
$++ A+ ++$	$Sp+ A+ ++ (1)$
$++ ++ B+$	$SpSp ++ B+ (1)$
$++ ++ ++$	$Sp+ ++ B+ (1)$

BACKCROSS OF F_1 HYBRIDS.

To the Swordtail

Melanotic hybrid \times Normal swordtail

$Sp+ A+ B+ \times ++ AA BB$

Backcross hybrids

NORMALS	MELANOTIC
$++ AA BB$	$Sp+ AA BB (4)$
$++ A+ BB$	$Sp+ AA B+ (3)$
$++ AA B+$	$Sp+ A+ BB (3)$
$++ A+ B+$	$Sp+ A+ B+ (2)$

Assuming that each dominant intensifying factor of the swordtail has the value of *one* (1) the degree of melanosis, or shift of the normal spotted pigment pattern to an intense blackening, is indicated by the values within the parentheses: *A* or *B* equals 1, together *A* and *B* equal 2, and so on.

When the melanotic (*Sp A B*) hybrid is backcrossed to the spotted platyfish (*Sp++*), the darkest backcross hybrids are rarely blacker than their melanotic parent which have an intensity value of *two* (2). There are four genotypic hybrids which have a melanotic intensity value of *one* (1), while two spotted genotypes have the normal pattern; these are listed under *normals*.

In the backcross to the swordtail, the severity of melanosis is enhanced in some backcross hybrids owing to intensity factors which reach the value of *four* (4) in 12½% of the brood; the value of *three* (3) in 25%; and the value of *two* (2) in 12½%. Fifty per cent. or remainder of the brood are normal, for they do not carry the essential *Sp* gene.

When the backcross hybrid members of this brood are again backcrossed to the swordtail, the results depend upon the specific genotype of the particular hybrid used. Hybrid *Sp+ AA BB* by *++ AA BB* swordtail produces black offspring essentially of the same intensity as its hybrid parent. Hybrid *Sp+ AA B+* by *++ AA BB* swordtail produces two grades of melanotic offspring while hybrid *Sp+ A+ B+* by *++ AA BB* swordtail produces three grades of melanotic

offspring. In this analysis it has been assumed that *A* has the same value as *B*. If modifying factors *A* and *B* had different values, or if there were more than two modifiers involved, far more divergent groups of hybrids might be expected.

In some of the matings between two swordtail species the degree of diversity in the coloration of various types of hybrids closely resembles, particularly in the first generation, the type of "blending" mentioned for "good" systematic characters. It was found that when the wild spotted type of *Xiphophorus montezumae* was mated with the wild *X. hellerii*, the degree of spotting in the hybrids fell to about 50% of normal; but when the weakly spotted hybrid was backcrossed to *X. hellerii*, the backcross offspring had hardly any spots at all. (These statements are based on Gordon's unpublished data.)

ORIGIN OF RED SWORDTAIL; A STUDY IN INTROGRESSION.

The example given by Hubbs (1940) to illustrate the distinction "between the systematic characters showing blending inheritance on one hand and the phase characters with a simple genetic basis on the other," concerns the origin of the red swordtail. (Incidentally, this is another good example of introgressive hybridization under domestication). According to Hubbs, "no red phase of the swordtail genus *Xiphophorus* [*hellerii*] has been taken in nature and none seems to have originated by mutation in captivity, but the red phase does occur in the related genus *Platypoecilus* [*maculatus*], which hybridizes rather freely with *Xiphophorus*. The hybrids produced by mating a *Xiphophorus* with a red *Platypoecilus* are, in part, of an undiluted red, though intermediate in the ordinary systematic characters, such as form, number of rays, and structure of the gonopodium. Red hybrids mating back to *Xiphophorus* produce three-quarter hybrids of which, however, a certain proportion is red. One or two backcrossings then reconstruct the swordtail, in all respects other than the red clothing."

First the comments concerning the coloration of the wild swordtail and wild platyfish may be discussed because subsequent color responses in their hybrids depend upon the interaction of *all* the color and color modifying genes involved (and possibly some of these genes may be linked to those affecting systematic characters). The wild swordtail has some red coloring of its own: a strong row of erythrophores runs along its lateral line in a zigzagging pattern. Also many red patches of grouped red cells are scattered in the dorsal fin. The red zigzag row of chromatophores of the wild *X. hellerii* is subject to modification; for instance, it may be intensified in a species hybrid by

genes contributed to the hybrid by a non-red species, *X. montezumae*.

Some wild platyfish may have light orange-red dorsal fins; some have brighter coloration about their throat and belly regions. But the reddest of the wild platyfish could not match the brilliant over-all red coloration of the commercially cultivated red platy. We must first explain the process by which the domesticated red platy got so red. The detailed explanation cannot be given adequately on this occasion, but briefly stated, there has been a reciprocal interchange of modifying genes—an example of *reciprocal introgressive hybridization*. The red swordtail has the red gene or genes of the platyfish, *Dr* or *Rt* or both plus its own modifiers of *Dr* and *Rt*. The process involved is essentially the same as that given for the *Sp* gene in this discussion. The brilliant red platyfish of the aquarist has the swordtail gene modifiers of *Dr* or *Rt* plus its own red genes. This has been attained by selection of the desirable combination on the part of the fish fanciers.

The fact that the red phase may have a number of genetic modifiers does not make it any less Mendelian in its mode of inheritance. It does indicate that the red characteristic is as sensitive to quantitative expression (which is the basis of the apparent blending inheritance) as characters that are often used in taxonomy.

In actual practice many subspecies are more easily distinguished on the basis of their distinctive colorations or color patterns than by slight average differences of body proportions or of skeletal elements. In the speciation process at the lowermost level of differentiation (and perhaps at somewhat higher levels as well) the evaluation of the patterns formed by pigment cells may be compared upon equal terms, at least, with patterns formed by bone, muscle or other groups of cells.

Just as we find a number of genetical systems controlling chromatic patterns in hybrids (for instance, one factor for stippling, *St*; or for *one-spot*, *O*; or for crescent complete, *Cc*; and for some others; two factors, *Co* and *E* for wagtail; three, for intense melanosis, and each dominant factor has a specific value, *Sp AA BB*, etc.) so other body structures may each have a special genetic basis and in addition there may be interactions between the genes in these restricted systems. In illustration of this latter point, Gerschler (1912), in studying the inheritance of the length of sword in swordtail-platyfish hybrids, found that at least three dominant factors were required to account for the results he obtained: *AA BB CC* where *AA* have the value of 2, *BB* have the value of 4 and *CC* have the value of 6. In addition, Gerschler found that the platyfish carries

dominant genes for some body features while the swordtail carries dominant genes for other groups of structures.

OTHER INSTANCES OF INTROGRESSION IN DOMESTICATED FISHES.

As indicated on several previous occasions, there are many good examples of introgressive hybridization in xiphophorin fishes—a complex of seven species. The papers of Kosswig (1929, 1937) and of his associate Breider (1937, 1938) contain detailed accounts of the behavior of specific genes of one species in the germ plasm of a related species. For the most part these are valid but many so-called *X. hellerii* genes such as *Mo*, *Rb* and *Sn* are all probably traceable to *P. maculatus*. Further discussion of these papers will be reserved for future occasions when related data on these specific color genes are presented. One comment may be made at this time: certain patterns of the platyfish have become associated so completely with the swordtail body form, and have been modified so thoroughly in the process, that part of their platyfish origin was not suspected. This is particularly true of the "*Mo*" character of the swordtail (*X. hellerii*) which now turns out to be due to the striped *Sr* pattern of wild platyfish. (This statement is based on unpublished data on the mating of wild platyfish of many different genotypes and wild swordtails.)

INTROGRESSION IN GENERAL.

In his treatment of natural polymorphism in relation to geographical variation, Mayr (1942) discusses the apparent rapid spread of a black mutation from one population having a high concentration of that particular mutation to other nearby populations where that mutation had been rare or absent. He states that this phenomenon, which looks like introgressive hybridization in geographical races, may possibly be explained on the basis of the great phylogenetic antiquity of certain alleles in related groups and species. In commenting upon the introgressive process, Mayr regards it as theoretical and unproved in animals. Anderson and Hubricht (1938) and other botanists have pointed out that introgressive hybridization is more likely to occur in localities which have come under man's management (or mismanagement) and in plants that may have escaped from cultivation. Dobzhansky (1941) states that while it is impossible to appraise the evolutionary role of introgressive hybridization, it may, in some organisms, result in the emergence of superior genotypes. For example of introgression in animals he cites the work of Boettger and of Franz on mollusks and that of Blair on toads and states that this phenomenon appears to be much more common in plants than in animals. It occurs

to me that if the origin of our domesticated animals were more precisely known many additional examples might be put forward in this group of organisms.

FISHES AND COTTON PLANTS.

Genetic conditions somewhat similar to those reported in this paper for xiphophorin fishes may be found among the species of cottons, studies upon which have been under way for many years by Harland (1936, 1939) and associated workers of the Imperial Cotton Growing Corporation, Hutchinson, Silow and Stephens. By planned hybridization between *Gossypium barbadense* and *G. hirsutum* and backcrossing of the hybrids to the original species, they have extracted hybrid compounds having predominantly the gene system of *G. barbadense* with a few genes of *G. hirsutum*, or conditions may be reversed. In the practical application of genetic research, plant and animal breeders are constantly trying to combine the desirable features of one organism with those of another. In some instances a high yielding plant is subject to a specific disease. A search is made among its wild relatives to find a disease-resistant strain and attempts are then made to combine the high yielding qualities of one with the disease-resisting qualities of the other. In effect, this, too, is introgression—if it is shown that the traits combined have a hereditary basis and separate origins.

GENES AND THE RATE OF DEVELOPMENT.

Changes in developmental rates by regulation of the external temperatures during specific periods of embryonic growth have altered the attainment of the usual number of vertebrae (and possibly dorsal fin-ray counts) in fishes. Hubbs and others have discovered this phenomenon; recently Gabriel (1944) has demonstrated it experimentally in developing *Fundulus*, and Gordon and Benzer (1945) have reviewed its significance in problems of speciation in xiphophorin fishes. Gabriel found that some strains of *Fundulus* were temperature labile, while others were genetically unalterable with regard to the number of vertebrae they attain.

In this connection, the work of Ford and Huxley (1929) on the attainment of definitive eye colors in *Gammarus* is suggestive. (A comprehensive discussion on the subject has recently been presented by Huxley, 1944). The eyes of the shrimp are usually black but mutants with eye colors of red, brown and dark brown are known. The eyes of the young stages of the normal shrimp are red but they gradually become darker. In the red-eyed mutant adult they never get darker; in other mutants they stop at the brown or darker stages. Huxley believes that genes produce their effect by influencing the rate of development of various substances

involved in pigment formation. This interpretation may be applied to the possible manner in which the modifying genes *A* and *B* of *X. hellerii* act upon the *Sp* gene of *P. maculatus* to produce melanosis in the F_1 hybrids ($Sp+ A+ B+$) and to produce severe melanosis in the backcross (to *X. hellerii*) hybrids ($Sp+ AA BB$). In the latter, the degree of melanosis which is usually attained by the F_1 hybrid in adult life is attained, according to Gordon (1937), in the backcross hybrid on the day of birth. A reversal of this trend may be seen in the inhibitory action of *X. hellerii* factors on the macromelanophore gene of *X. montezumae*.

The concept of the influence of genes on the rate of development has been used by Goldschmidt for the interpretation of sex attainment and other features. It may also apply, possibly, to vertebrae number, fin-ray count and other "taxonomic" characters.

SUMMARY.

1. The Mexican platyfish gene PC^o , one of seven dominant, multiple, autosomal alleles, produces a simple comet-like pattern in the tail fin in wild *Platypoecilus maculatus*. Representatives of the comet gene PC^o are found in natural populations of the platyfish in the Rio Jamapa and Rio Papaloapan but not in the Rio Coatacoalcos and the Rio Usumacinta.

2. When a "wild" *P. maculatus* carrying PC^o is mated with "domesticated" platyfish or with "wild" *Xiphophorus hellerii* the phenotype of the hybrids show all the fins considerably darker, producing a new variety termed the *wagtail*. The modified effect of PC^o from the comet to the wagtail is due to a specific factor *E* which has no visible effect of its own. *E* is autosomal and independent of the P^O , PM , PM^c , PC^o , PC , PC^c , PT series and of *st*.

3. It is suggested that "domesticated" stock of *P. maculatus* acquired the *E* factor by a process of introgression.

4. The Platyfish gene PC^o was transferred by hybridization to platyfish-swordtail hybrids. By a series of backcrosses of the hybrids to the swordtail, fish were bred having the configuration of *Xiphophorus hellerii* but containing PC^o of *Platypoecilus maculatus*. This new variety is called the wagtail swordtail among fish fanciers. The establishment of this new stock is an example of introgressive hybridization under conditions of domestication. Hybridization under natural conditions has not been known to take place.

5. The domesticated red swordtail of aquarists is considered and its origin is traced to "red" gene of *P. maculatus* modified by intensification by at least two *X. hellerii* genes. These effects resemble the behavior of taxonomic characters in species crosses. Taxonomic characters at the lower-

most levels, sub-species and perhaps species, are compared genetically with color pattern characters.

6. A number of genetic similarities and parallelisms of introgressive hybridization in cotton plants and xiphophorin fishes are indicated.

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EXPLANATION OF THE PLATES.

PLATE I.

Fig. 1. The wild color types in natural populations.

There are many pools in Mexico, particularly in the Rio Papaloapan drainage area, in which most or all of the color types shown here may be found living side by side.

The first column represents the two forms of the swordtail (*Xiphophorus hellerii*). The upper one represents a weakly spotted type which is very rare, occurring in wild populations in a frequency of less than one-half of one per cent.

The second column represents the tail markings of the platyfish (*Platypoecilus maculatus*). These form a series of autosomal, dominant, multiple alleles. Reading from top to bottom: one-spot, *PO*; twin-spot, *PT*; single crescent, *PC*; complete crescent, *PCc*; moon, *PM*; moon complete, *PMc*; and comet, *PCo*. There is a universal recessive shown at the top of the next column, usually referred to as the + gene. Of all the seven dominant alleles, only the comet, *PCo*, reacts to the presence of the *E* modifier.

The third column shows, in addition to the universal recessive, on top, the five sex-linked patterns: striped-sided, spot-sided, spotted dor-

sal, black-bottomed and the black-banded. These patterns are formed by macromelanophores. The series in the column to the left are formed by micromelanophores.

The fourth column shows the three red colored varieties: the red-dorsal, the red anal-fin and the bleeding heart. These are sex-linked and are formed by erythrophores.

Fig. 2. *The Introgression of the Platyfish Gene Comet (Co) Into the Germ Plasm of the Sword-tail.*

The series of matings shown in this chart begins in the upper left hand corner. A wild swordtail (*Xiphophorus hellerii*) is mated with a wild comet-marked platyfish (*Platyopocilus maculatus*). The mating of these distinct species is effective under conditions of domestication in an aquarium where one female and one male are placed in a single container. Under conditions in nature, these two species, which are often found living side by side, apparently do not hybridize. In more than 10,000 specimens caught in nature, and examined, no hybrids were found between them.

The "domesticated" hybrids are shown in the lower left. The comet pattern of the platyfish is transformed into the wagtail; note the shortening of the sword compared with its normal length in male wild swordtails shown elsewhere in the chart. There is a blend of body configurations and some color patterns in the hybrids.

If one of the wagtail platyfish-swordtail hybrids is selected and mated to a pure wild swordtail male as indicated in the pair in the lower right hand corner, the body contours, the length of the tail and other features in some of the resulting offspring resemble that of the wild swordtail, but the wagtail pattern in their make-up is a sign of the fact that at least one gene, the comet gene (*Co*) of the platyfish, is present in the domesticated and reconstructed swordtail. Thus the presence of a platyfish gene in the germ plasm of the swordtail is a result of introgressive hybridization.

PLATE II.

Fig. 3. *The Development of the Golden Wagtail Platyfish.*

The golden wagtail platyfish was developed by mating a domesticated golden platyfish with a wild, gray-green comet. In the first generation all the offspring were gray-green, owing to the fact that the wild type gene *St* for micromelanophores is dominant to the golden, or non-micromelanophore type. The wagtail reaction is evident in the F_1 owing to the interaction of the wild comet gene (*Co*) with its modifier *E*. When the gray-green wagtail F_1 are inbred, six phenotypes appear in the next generation. Reading down by columns, the column on the extreme left: gray, comet; gray; golden, comet. The second column: gray, wagtail; golden; golden, wagtail. The third and fourth columns represent

the males and the number of phenotypes in them is the same as in the females just enumerated.

When two golden wagtails are selected from the second generation offspring and mated, if by chance these individuals were homozygous for the comet (*Co*) and for the extensor of comet gene (*E*), then a pure breeding line of golden wagtail platyfish may be established as indicated in the chart.

The presence of the modified gene *E* in domesticated platyfish may be explained by suggesting that *E* is found in natural populations of the related species, the swordtail (*Xiphophorus hellerii*), and it has infiltrated by introgressive hybridization into the germ plasm of the domesticated platyfish (*Platyopocilus maculatus*).

PLATE III.

Fig. 4. *The Early and the Perfected Wagtail Platyfish.*

In some of the early matings between the "wild" comet platyfish (*Platyopocilus maculatus*) with the "domesticated" strains, the red, black-spotted type of female was used. In some instances the *R* and *Sp* genes were carried by the *W* chromosomes, thus producing red, black-spotted daughters and recessive, gray sons. One of the spotted daughters and one of the gray males are shown in this plate facing to the right; both of them carry the modified form of the comet pattern known as wagtail. The fish facing to the left was obtained by use of the golden domesticated strain and a wild comet platy. It represents one of the second generation young, in which the color is bright yellow, but the fins are blackened by the interaction of the comet gene (*Co*) with a modifier, *E*, the extensor of the comet.

The wagtail pigmentation reaction involves more than the deepening color of the fins: the snout and parts of the operculum are blackened by small melanophores. These pigmented cells are found in the integumentary tissues surrounding the region of the premaxillaries; they are found in the tissues surrounding the region of the dentary and continue from this point caudally along a line formed by the ventral margin of the articular, quadrate and preopercular; then the line forms an upwardly directed angle following the posterior margin of the preopercular. Photographs by S. C. Dunton.

Fig. 5. *Wagtail Swordtails.*

This pair of swordtails have all the external attributes of swordtails, *Xiphophorus hellerii*, except for the black coloring of the fins. This color pattern is the result of the interaction of a platyfish gene comet (*Co*) with a specific modifier (*E*). These swordtails are the product of introgressive hybridization. In the course of their genetic history, a *Platyopocilus maculatus* gene, *Co*, has been incorporated into the germ plasm of the swordtail. Photographs by S. C. Dunton.

WILD COLOR TYPES CAUGHT IN THE SAME POOL IN MEXICO

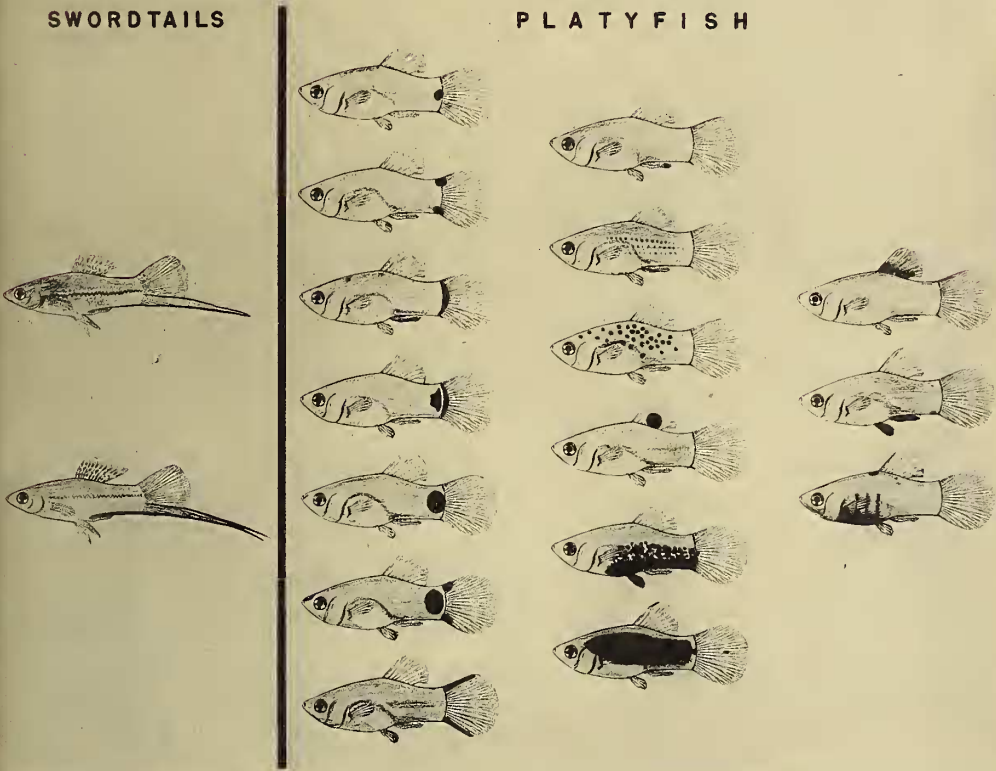


FIG. 1.

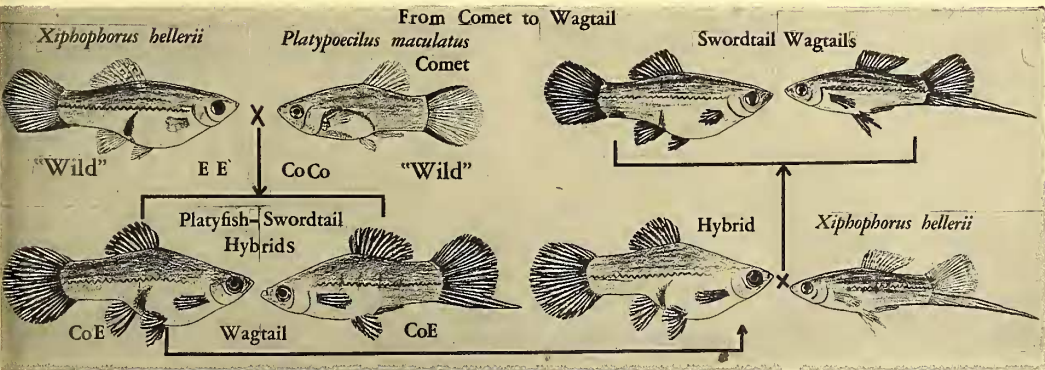


FIG. 2.

INTROGRESSIVE HYBRIDIZATION IN DOMESTICATED FISHES.
I. THE BEHAVIOR OF COMET—A *PLATYPOECILUS MACULATUS* GENE IN *XIPHOPHORUS HELLERII*.

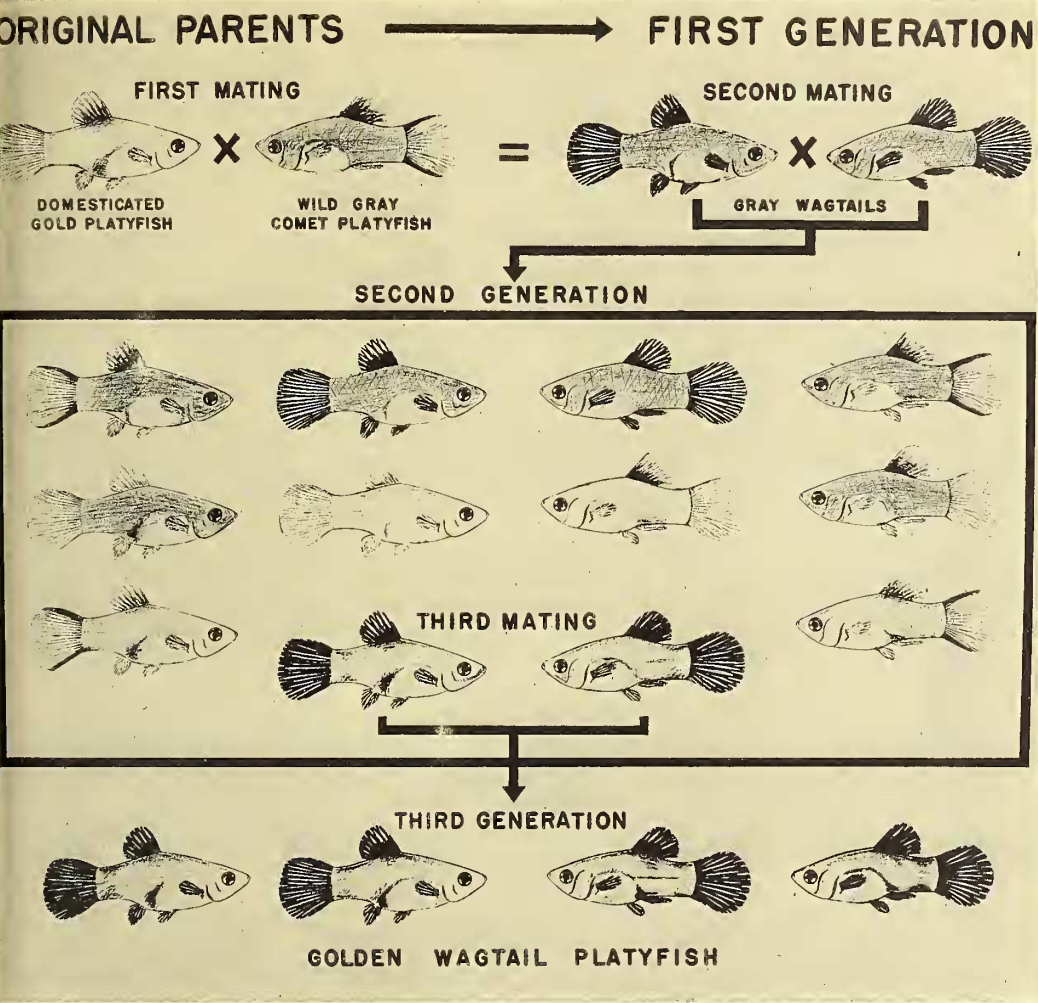


FIG. 3.

INTROGRESSIVE HYBRIDIZATION IN DOMESTICATED FISHES.

I. THE BEHAVIOR OF COMET—A PLATYPOECILUS MACULATUS GENE IN XIPHOPHORUS HELLERII.



FIG. 4.

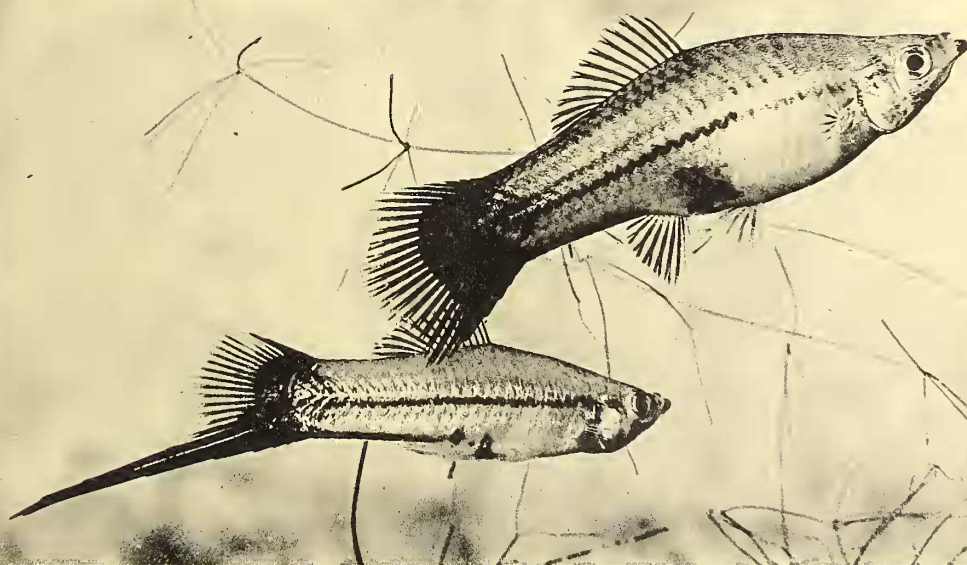


FIG. 5.

INTROGRESSIVE HYBRIDIZATION IN DOMESTICATED FISHES.
I. THE BEHAVIOR OF COMET—A *PLATYPOECILUS MACULATUS* GENE IN *XIPHOPHORUS HELLERII*.